Article

Expression Characteristics and Functional Analysis of the *ScWRKY3* Gene from Sugarcane

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Supplementary Materials

Table S1. Primary structure analysis of ScWRKY3

Primary structure characteristics	Prediction results
Number of amino acids	236
Molecular weight	25979.45 Da
Theoretical isoelectric point (pI)	8.58
Grand average of hydropathicity (GRAVY)	-0.492
The instability index (II)	56.08

The primary structure of the ScWRKY3 protein was predicted by ProtParam (https://web.expasy.org/protparam/).

Table S2. Primers used in this study

Primer name	Primer sequence (5′-3′)	Usage
ScWRKY3-F	ACCACCACTAACCCCAAAGC	Full length amplification
ScWRKY3-R	CTGCGTCGTCAATGTATGCG	Full length amplification
ScWRKY3-QF	GCGACGTGGATGTACTGGATGA	qRT-PCR analysis
ScWRKY3-QR	CCTTGGATGGAGGCTGTTCTTG	qRT-PCR analysis
GAPDH-F	CACGGCCACTGGAAGCA	qRT-PCR analysis
GAPDH-R	TCCTCAGGGTTCCTGATGCC	qRT-PCR analysis
ScWRKY3-Gate-F	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u>AT GCAGGCATATATGGAGGG	Gateway entry vector construction and semi-quantitative PCR analysis
ScWRKY3-Gate-R	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTC</u>GAA GGAGCTGAAGCAATCGG	Gateway entry vector construction and semi-quantitative PCR analysis
ScWRKY4-Gate-F	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u>AT GGAGGGGAGCAGCCAGCT	Gateway entry vector construction
ScWRKY4-Gate-R	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTC</u>GAG CGACGTGAAAGCGCAGC	Gateway entry vector construction
ScWRKY3-BD-F	<u>GGAATTCCATATG</u>ATGCAGGCATATATGGAGGG	Bait vector construction
ScWRKY3-BD-R	<u>CGGGATCC</u>GAAGGAGCTGAAGCAATCGG	Bait vector construction
ScWRKY3-AD-F	<u>GGAATTCCATATG</u>ATGCAGGCATATATGGAGGG	Prey vector construction
ScWRKY3-AD-R	<u>CGGGATCC</u>GAAGGAGCTGAAGCAATCGG	Prey vector construction
ScWRKY4-BD-F	<u>GGAATTCCATATG</u>ATGGAGGGGAGCAGCCAGCT	Bait vector construction
ScWRKY4-BD-R	<u>CGGGATCC</u>GAGCGACGTGAAAGCGCAGC	Bait vector construction
ScWRKY4-AD-F	<u>CCGGAATTC</u>ATGGAGGGGAGCAGCCAGCT	Prey vector construction
ScWRKY4-AD-R	<u>CCGCTCGAG</u>GAGCGACGTGAAAGCGCAGC	Prey vector construction
NtH201-F	CAGCAGTCCTTTGGCGTTGTC	qRT-PCR analysis
NtH201-R	GCTCAGTTTAGCCGCAGTTGTG	qRT-PCR analysis
NtH203-F	TGGCTCAACGATTACGCA	qRT-PCR analysis
NtH203-R	GCACGAAACCTGGATGG	qRT-PCR analysis
NtH515-F	TTGGGCAGAATAGATGGGTA	qRT-PCR analysis
NtH515-R	TTTGGTGAAAGTCTTGGCTC	qRT-PCR analysis
NtNPR1-F	GGCGAGGAGTCCGTTCTTTAA	qRT-PCR analysis
NtNPR1-R	TCAACCAGGAATGCCACAGC	qRT-PCR analysis
NtPR1a/c-F	AACCTTTGACCTGGGACGAC	qRT-PCR analysis
NtPR1a/c-R	GCACATCCAACACGAACCGA	qRT-PCR analysis
NtPR2-F	TGATGCCCTTTTGGATTCTATG	qRT-PCR analysis
NtPR2-R	AGTTCCTGCCCCGCTTT	qRT-PCR analysis
NtPR3-F	CAGGAGGGTATTGCTTTGTTAGG	qRT-PCR analysis
NtPR3-R	CGTGGGAAGATGGCTTGTTGTC	qRT-PCR analysis
NtEFE26-F	CGGACGCTGGTGGCATAAT	qRT-PCR analysis
NtEFE26-R	CAACAAGAGCTGGTGCTGGATA	qRT-PCR analysis
NtAccdeaminase-F	TCTGAGGTTACTGATTTGGATTGG	qRT-PCR analysis
NtAccdeaminase-R	TGGACATGGTGGATAGTTGCT	qRT-PCR analysis
NtEF-1α-F	TGCTGCTGTAACAAGATGGATGC	qRT-PCR analysis
NtEF-1α-R	GAGATGGGGACAAAGGGGATT	qRT-PCR analysis

attB1 and *attB2* adapters are underlined in the forward primers and in the reverse primers for gateway entry vector construction, respectively. The added restriction enzyme sites of *Eco*R I (GAATTC), *Xho* I (CTCGAG), *Nde* I (CATATG)

and *Bam*H I (GGATCC) are underlined in the primers for bait or prey vectors construction, and the double underlined areas in these primers indicate protective bases.

Table S3. Raw calculations of tissue-specific expression of *ScWRKY3* in different 10-month-old ROC22 tissues by qRT-PCR

Tissues	$2^{-\Delta\Delta C_T}$	Standard error
R	1.02874	0.17959
B	4.70889	1.64113
L	1.13441	0.35043
SP	29.17377	4.75581
SE	2.50973	0.36618

The tissues (root, bud, leaf, stem pith, and stem epidermis) are represented by R, B, L, SP, and SE, respectively. Data are normalized to the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression level. All data points are means ± standard error (*n* = 3). Bars superscripted by different lowercase letters indicate significant differences, as determined by Duncan's new multiple range test (p-value < 0.05).

Table S4. Raw calculations of gene expression patterns of *ScWRKY3* in 4-month-old ROC22 plantlets under abiotic stress

Treatment time	NaCl		PEG	
	$2^{-\Delta\Delta C_T}$	Standard error	$2^{-\Delta\Delta C_T}$	Standard error
0 h	1.02248	0.15162	1.00235	0.04850
0.5 h	1.12695	0.03042	0.67118	0.05745
3 h	1.10801	0.06703	38.57182	10.9277
6 h	0.63938	0.08744	1.27614	0.22401
24 h	3.27612	0.26971	1.11389	0.12550

NaCl, sodium chloride (simulating salt stress) (250 mM); PEG, polyethylene glycol (simulating drought treatment) (25.0%). Data are normalized to the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression level. All data points are means ± standard error (*n* = 3). Bars superscripted by different lowercase letters indicate significant differences, as determined by Duncan's new multiple range test (p-value < 0.05).

Table S5. Raw calculations of gene expression of *ScWRKY3* in 4-month-old ROC22 plantlets under plant hormone stress

Treatment time	SA		MeJA		ABA	
	$2^{-\Delta\Delta C_T}$	Standard error	$2^{-\Delta\Delta C_T}$	Standard error	$2^{-\Delta\Delta C_T}$	Standard error
0 h	1.00130	0.00001	1.00217	0.00011	1.00515	0.08640
3 h	0.39460	0.01486	0.55133	0.00048	0.80955	0.04149
6 h	0.59523	0.10924	0.42110	0.05784	1.66832	0.15545
24 h	0.49914	0.00962	0.33474	0.08544	0.80563	0.11352

SA, salicylic acid (5 mM); MeJA, methyl jasmonate (25 μM); ABA, abscisic acid (100 μM). Data are normalized to the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression level. All data points are means ± standard error (*n* = 3). Bars superscripted by different lowercase letters indicate significant differences, as determined by Duncan's new multiple range test (p-value < 0.05).

Table S6. Raw calculations of expression of the *ScWRKY3* gene after infection with smut pathogen

Treatment time	Yacheng05-179		ROC22	
	$2^{-\Delta\Delta C_T}$	Standard error	$2^{-\Delta\Delta C_T}$	Standard error
0 h	1.02011	0.25922	1.00740	0.08826
24 h	1.26167	0.47474	0.50795	0.06088
48 h	1.13023	0.09219	0.95991	0.00450
72 h	1.05532	0.10303	0.79783	0.03618

Yacheng05-179 is a smut-resistant *Saccharum* hybrid cultivar, and ROC22 is a smut-susceptible *Saccharum* hybrid cultivar. Data are normalized to the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression level. All data points are means ± standard error (*n = 3*). Bars superscripted by different lowercase letters indicate significant differences, as determined by Duncan's new multiple range test (p-value < 0.05).

Table S7. Raw calculations of the transcript level of nine immunity-associated marker genes in the *Nicotiana benthamiana* leaves after one day of agroinfiltration

Gene name	35::00		35::ScWRKY3	
	$2^{-\Delta\Delta C_T}$	Standard error	$2^{-\Delta\Delta C_T}$	Standard error
NtHSR201	1.00035	0.01878	0.31325	0.01010
NtHSR203	1.02856	0.16092	5.55995	0.26401
NtHSR515	1.01574	0.12341	3.05449	0.03158
NtPR1	1.02067	0.15147	14.15632	0.80030
NtPR-1a/c	1.00025	0.01561	0.83799	0.01543
NtPR2	1.00014	0.01178	0.99941	0.02160
NtPR3	1.00096	0.03072	8.79463	0.34939
NtEFE26	1.00027	0.01638	1.46628	0.01135
NtAccdeaminase	1.00442	0.06553	2.35996	0.06627

Data are normalized to the *NtEF-1α* expression level. All data points are means ± standard error (*n = 3*). Bars superscripted by different lowercase letters indicate significant differences, as determined by Duncan's new multiple range test (p-value < 0.05).

Table S8. Raw calculations of the transcripts of nine immunity-associated marker genes in the *Nicotiana benthamiana* leaves after inoculation with *Ralstonia solanacearum* for one day and seven days

Gene name	Treatment time	35::00		35::ScWRKY3	
		$2^{-\Delta\Delta C_T}$	Standard error	$2^{-\Delta\Delta C_T}$	Standard error
NtH201	0 d	1.00035	0.01878	1.03671	0.18533
	1 d	23.83326	0.44415	1.00248	0.05525
	7 d	21.64681	0.79454	1.00289	0.04423
NtH203	0 d	1.02856	0.16092	1.00226	0.04759
	1 d	8.47359	0.08132	2.51027	0.04509
	7 d	1.13105	0.03165	3.32439	0.10266
NtH515	0 d	1.01574	0.12341	1.00011	0.01034
	1 d	23.12115	0.75118	2.70385	0.15356
	7 d	7.13494	0.12217	2.65545	0.04517
NtNPR1	0 d	1.02067	0.15147	1.00310	0.05671
	1 d	48.32176	3.78460	24.73383	3.91994
	7 d	26.22899	0.03695	12.70940	1.69010
NtPR1a/c	0 d	1.00025	0.01561	1.00087	0.02918
	1 d	2.69564	0.19104	2.23772	0.04028
	7 d	7.34399	0.43130	1.03147	0.03602
NtPR2	0 d	1.00014	0.01178	1.43645	0.85614
	1 d	0.00540	0.00130	0.00003	0.00001
	7 d	6.91943	1.35432	5.07464	0.76171
NtPR3	0 d	1.00096	0.03072	1.00158	0.03979
	1 d	1.17475	0.02933	0.78957	0.29541
	7 d	4.22494	0.65946	1.95949	0.16057
NtEFE26	0 d	1.00027	0.01638	1.00006	0.00774
	1 d	0.80520	0.01612	0.29558	0.00401
	7 d	0.20565	0.00213	0.07477	0.00183
NtAccdeaminase	0 d	1.00442	0.06553	1.00078	0.02811
	1 d	0.60119	0.01118	0.63861	0.02083
	7 d	0.29504	0.01311	0.63590	0.00549

Data are normalized to the *NtEF-1α* expression level. All data points are means ± standard error (*n* = 3). Bars superscripted by different lowercase letters indicate significant differences, as determined by Duncan's new multiple range test (p-value < 0.05).

Table S9. Raw calculations of the transcripts of nine immunity-associated marker genes in the *Nicotiana benthamiana* leaves after inoculation with *Fusarium solani* var. *coeruleum* for one day and seven days

Gene name	Treatment time	*35::00*		*35::ScWRKY3*	
		$2^{-\Delta\Delta C_T}$	Standard error	$2^{-\Delta\Delta C_T}$	Standard error
NtH201	0 d	1.00195	0.04430	1.00045	0.02137
	1 d	0.27135	0.01817	0.53091	0.01105
	7 d	0.05003	0.00352	0.14749	0.00262
NtH203	0 d	1.00208	0.04575	1.00036	0.01894
	1 d	0.67138	0.03458	0.42983	0.03520
	7 d	0.11990	0.00492	0.26738	0.00426
NtH515	0 d	1.01527	0.11926	1.02883	0.16165
	1 d	13.54125	0.28097	0.00261	0.00001
	7 d	1.19530	0.00971	0.00024	0.00002
NtNPR1	0 d	1.00195	0.05101	1.02043	0.16589
	1 d	6.91331	0.45229	1.99066	0.30924
	7 d	3.44026	0.03078	0.20613	0.02542
NtPR1a/c	0 d	1.00025	0.01561	1.00054	0.02319
	1 d	0.22801	0.04156	0.10524	0.00300
	7 d	0.99548	0.02271	0.27953	0.00774
NtPR2	0 d	1.00542	0.07383	1.02190	0.14310
	1 d	1.40590	0.24153	1.22961	0.13109
	7 d	0.04811	0.00295	0.64970	0.12044
NtPR3	0 d	1.00096	0.03072	1.00158	0.03979
	1 d	0.04353	0.00743	0.27462	0.03474
	7 d	0.80819	0.09846	2.35022	0.14629
NtEFE26	0 d	1.00077	0.02752	1.00006	0.00774
	1 d	0.00036	0.00002	0.00126	0.00006
	7 d	0.00021	0.02002	0.00032	0.00004
NtAccdeaminase	0 d	1.00442	0.06553	1.00078	0.02811
	1 d	0.13969	0.03101	1.68063	0.04270
	7 d	0.04792	0.00090	0.15812	0.03980

Data are normalized to the *NtEF-1α* expression level. All data points are means ± standard error (*n* = 3). Bars superscripted by different lowercase letters indicate significant differences, as determined by Duncan's new multiple range test (p-value < 0.05).

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ROC22-ScWRKY3      ACCACCACTAACCCCAAAGCATCATCGTCATCGTCAACCTTATCGTGCACAGTTATTGACACCAAAAGGAGAGAGATGCA    80
YC05-179-ScWRKY3   ACCACCACTAACCCCAAAGCATCATCGTCATCGTCAACCTTATCGTGCACAGTTATTGACACCAAAAGGAGAGAGATGCA    80
Consensus          accaccactaaccccaaagcatcatcgtcatcgtcaaccttatcgtgcacagttattgacaccaaaaggagagagatgca

ROC22-ScWRKY3      TTCCGATCCATCCTGCGAATTTTCTGCCCCGGCCGCCCAACCAAGATCCACACTTTGATCATCAATCGATCGCTAGCGCA    160
YC05-179-ScWRKY3   TTCCGATCCATCCTGCGAATTTTCTGCCCCGGCCGCCCAACCAAGATCCACACTTTGATCATCAATCGATCGCTAGCGCA    160
Consensus          ttccgatccatcctgcgaattttctgccccggccgcccaaccaagatccacactttgatcatcaatcgatcgctagcgca

ROC22-ScWRKY3      TATGCAGGCATATATGGAGGGAGGCCAGTTGAGTGCTTGCCTTCCTAGCTTCCTTGTGCCGGATCACTACGCCGGCTTCC    240
YC05-179-ScWRKY3   TATGCAGGCATATATGGAGGGAGGCCAGTTGAGTGCTTGCCTTCCTAGCTTCCTTGTGCCGGATCACTACGCCGGCTTCC    240
Consensus          tatgcaggcatatatggagggaggccagttgagtgcttgccttcctagcttccttgtgccggatcactacgccggcttcc

ROC22-ScWRKY3      CCCTTCCTCTCCCGCTACAACTTCCTAGCCAACCAAACAACAAGCTTTTCCAGATGCCGTTTGTAGTTAACCAGGAAGAG    320
YC05-179-ScWRKY3   CCCTTCCTCTCCCGCTACAACTTCCTAGCCAACCAAACAACAAGCTTTTCCAGATGCCGTTTGTAGTTAACCAGGAAGAG    320
Consensus          cccttcctctcccgctacaacttcctagccaaccaaacaacaagcttttccagatgccgtttgtagttaaccaggaagag

ROC22-ScWRKY3      ACAGAGAACCATGGCGGCATGCTCTCCTCCGACCATTGTGGTGGACTATACCCGCTGCCGGCACTGCCCTTCGGCAGCTG    400
YC05-179-ScWRKY3   ACAGAGAACCATGGCGGCATGCTCTCCTCCGACCATTGTGGTGGACTATACCCGCTGCCGGCACTGCCCTTCGGCAGCTG    400
Consensus          acagagaaccatggcggcatgctctcctccgaccattgtggtggactatacccgctgccggcactgcccttcggcagctg

ROC22-ScWRKY3      CTCCGGCGCCGCCGCCGCAACAGCATGCGGTGGGAAGCCGACGGCCGGTTTCATGCCCAGTGCTATTGGCGCTGAGGAGG    480
YC05-179-ScWRKY3   CTCCGGCGCCGCCGCCGCAACAGCATGCGGTGGGAAGCCGACGGCCGGTTTCATGCCCAGTGCTATTGGCGCTGAGGAGG    480
Consensus          ctccggcgccgccgccgcaacagcatgcggtgggaagccgacggccggtttcatgcccagtgctattggcgctgaggagg

ROC22-ScWRKY3      TCTGCACCTCGGTGGCTACTAAACTAGGTTGCAACGAGAGTAATAGCACATGGTGGAAGGGCTCAGCAGCTACGACTGCG    560
YC05-179-ScWRKY3   TCTGCACCTCGGTGGCTACTAAACTAGGTTGCAACGAGAGTAATAGCACATGGTGGAAGGGCTCAGCAGCTACGACTGCG    560
Consensus          tctgcacctcggtggctactaaactaggttgcaacgagagtaatagcacatggtggaagggctcagcagctacgactgcg

ROC22-ScWRKY3      GAGAGAGGGAAGATGAAGGTGAGGAGGAAGATGAGGGAACCGAGGTTTTGCTTCCAGACCAGAAGCGACGTGGATGTACT    640
YC05-179-ScWRKY3   GAGAGAGGGAAGATGAAGGTGAGGAGGAAGATGAGGGAACCGAGGTTTTGCTTCCAGACCAGAAGCGACGTGGATGTACT    640
Consensus          gagagagggaagatgaaggtgaggaggaagatgagggaaccgaggttttgcttccagaccagaagcgacgtggatgtact

ROC22-ScWRKY3      GGATGATGGCTACAAGTGGAGGAAGTACGGGCAGAAGGTTGTCAAGAACAGCCTCCATCCAAGGAGCTATTTCCGGTGCA    720
YC05-179-ScWRKY3   GGATGATGGCTACAAGTGGAGGAAGTACGGGCAGAAGGTTGTCAAGAACAGCCTCCATCCAAGGAGCTATTTCCGGTGCA    720
Consensus          ggatgatggctacaagtggaggaagtacgggcagaaggttgtcaagaacagcctccatccaaggagctatttccggtgca

ROC22-ScWRKY3      CTCACAGCAACTGCCGCGTGAAGAAACGGGTGGAGCGGCTGTCGACGGACTGCCGCATGGTGATGACCACGTACGAGGGC    800
YC05-179-ScWRKY3   CTCACAGCAACTGCCGCGTGAAGAAACGGGTGGAGCGGCTGTCGACGGACTGCCGCATGGTGATGACCACGTACGAGGGC    800
Consensus          ctcacagcaactgccgcgtgaagaaacgggtggagcggctgtcgacggactgccgcatggtgatgaccacgtacgagggc

ROC22-ScWRKY3      CGCCACACGCACTCTCCCTGCAGCGACGACGCTTCCTCCGCCGACCACACCGATTGCTTCAGCTCCTTCTGAATCTATCT    880
YC05-179-ScWRKY3   CGCCACACGCACTCTCCCTGCAGCGACGACGCTTCCTCCGCCGACCACACCGATTGCTTCAGCTCCTTCTGAATCTATCT    880
Consensus          cgccacacgcactctccctgcagcgacgacgcttcctccgccgaccacaccgattgcttcagctccttctgaatctatct

ROC22-ScWRKY3      ATCCCACGACCGCATACATTGACGACGCAG                                                    910
YC05-179-ScWRKY3   ATCCCACGACCGCATACATTGACGACGCAG                                                    910
Consensus          atcccacgaccgcatacattgacgacgcag
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Figure S1. Nucleic acid sequences alignment of *ScWRKY3* in ROC22 and Yacheng05-179. The black color indicates the homology level of conservation of the nucleic acid sequences in the alignment at 100%.
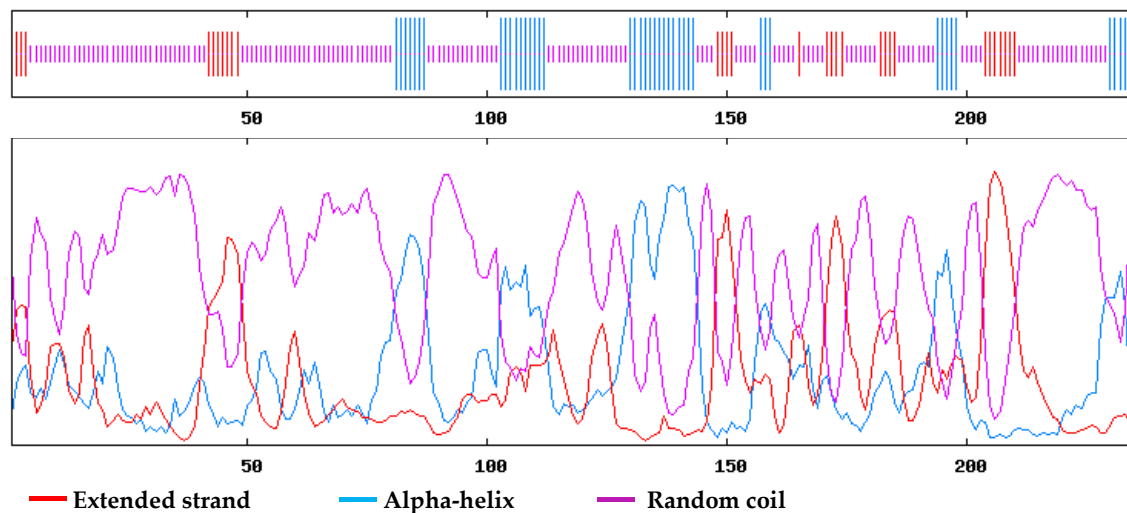
Figure S2. Secondary structure prediction of ScWRKY3. NPS@ server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hnn.html) is used to predict this structure.
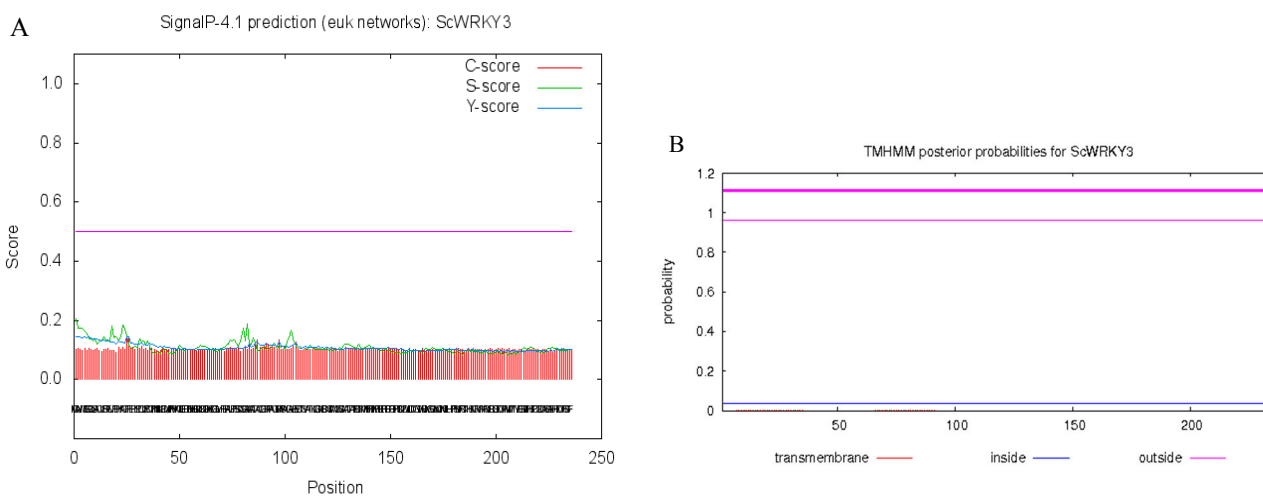

Figure S3. Signal peptide and transmembrane domain prediction of ScWRKY3. (A) SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) is used to predict the signal peptide of ScWRKY3. (B) TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) is used to predict the transmembrane domain of ScWRKY3.

Figure S4. Subcellular localization prediction of ScWRKY3. Euk-mPLoc 2.0 Server (http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/) is used to predict the subcellular localization of ScWRKY3.

A

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ScWRKY3    MQAYMEGGQLSACLPSFLVPDHYAGFPLPLPLQLPSQPNNKLFQMPFVVNQE.ETENHGG..MLSSDHCGGLYPLPALPFGSCSGAA.AA    86
SbWRKY57   MQAYMEGGQLSACLPGFLVPDHYA.FPLPLPLQLPSSQ.NKLFQMPFVVDQEAETENHGGGGMLSSDHCG.LYPLPALPFGSCSGAACAA    87
Consensus  mqaymeggqlsaclpgflvpdhyagfplplplqlpsqpnnklfqmpfvvdqeaetenhggggmlssdhcgglyplpalpfgscsgaagaa

ScWRKY3    TACGGKPTAGFMPSAIGAEEVCTSVATKLGCNESNSTWWKGSAATT.AERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQKVVK    175
SbWRKY57   TACGGKPTAGFMPSAIVAEVCTSVTKLGCNDSNGTWWKGSAATTIAERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQKVVK    177
Consensus  tacggkptagfmpsaigaeevctsvatklgcndsngtwwkgsaattiaergkmkvrrkmreprfcfqtrsdvdvlddgykwrkygqkvvk

ScWRKY3    NSLHPRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSADHTDCFSSF    236
SbWRKY57   NSLHPRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSADHTDCFTSF    238
Consensus  nslhprsyfrcthsncrvkkrverlstdcrmvmttyegrhthspcsddassadhtdcfssf
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B

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ScWRKY3    MQAYMEGGQLSACLPSFLVPDHYAGFPLPLPLQLPSQPNNKLFQMPFVVNQEETENHGGMLSSDHCGGLYPLPALPFGSCSGAAAATACG    90
MlWRKY12   ....MEGGQLSACLPGFFVPDHYAAFPLPLPLQLPSQPNNKLFQMPFVVDQEETENHGGMLSSDHCGGLYPLPALPFVSSGAATATACG    86
Consensus  mqaymeggqlsaclpgffvpdhyaafplplplqlpsqpnnklfqmpfvvdqeetenhggmlssdhcgglyplpalpfgscsgaaaatacg

ScWRKY3    GKPTAGFMPSAIGAEEVCTSVATKLGCNESNSTWWKGSAATT.AERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQKVVKNSLH    179
MlWRKY12   GKPTAGFMPSTIGAEEVCTSVTKLGCNESNSTWWKGSAATIAERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQKVVKNSLH    176
Consensus  gkptagfmpsaigaeevctsvatklgcnesnstwwkgsaaatiaergkmkvrrkmreprfcfqtrsdvdvlddgykwrkygqkvvknslh

ScWRKY3    PRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSADHTDCFSSF    236
MlWRKY12   PRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSCDHTDCFSSF    233
Consensus  prsyfrcthsncrvkkrverlstdcrmvmttyegrhthspcsddassadhtdcfssf
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C

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ScWRKY3    MQAYMEGGQLS.A.CLPSFLVPDHYAGFPLPLPLQLPSQPNNK.LFQMPFVVNQEETENHGGMLSSDHCGGLYPLPALPFGSCSGAAAAT    87
ZmWRKY51   MQAYMEGGQLLGACCLPGFLVPDHYAAFPLPLPLQLPSQPNDNRLFQMPFD..QEEAENHGGMIYSDCCGLYPLPA.FGSCSGAAAGAT    85
Consensus  mqaymeggqllgacclpgflvpdhyaafplplplqlpsqpndkrlfqmpfdvnqeeaenhggmlssdhcgglyplpalpfgscsgaaaaat

ScWRKY3    ACGGKPTAGFMPSAIGAEE.VCTSVATKLGCNESNSTWWKGSAATTAERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQKVVKN    176
ZmWRKY51   A...KPTAGFMPSTIGAETKVCTSLTTK.GCNESNSTWWKGSAATIAERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQKVVKN    171
Consensus  acggkptagfmpsaigaeekvctslatklgcnesnstwwkgsaatiaergkmkvrrkmreprfcfqtrsdvdvlddgykwrkygqkvvkn

ScWRKY3    SLHPRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSADHTDCFSSF    236
ZmWRKY51   SLHPRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSGDHTDCFSSF    231
Consensus  slhprsyfrcthsncrvkkrverlstdcrmvmttyegrhthspcsddassadhtdcfssf
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D

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ScWRKY3    MQAYMEGGQLSACLPSFLVPDHYAGFPLPLPLQLPSQPNNKLFQMPFVVNQEETENHGGMLSSDHCGGLYPLPALPFGSCSGAAAATACG    90
SiWRKY12   MQAYMEGGQLGACLPSFLVPDHYA.FPLPLPLQLPSQP.NKLFQMPFD..QEESENHGVMLSSDHC.GLYQLPALPLC.CSGAASAVACG    84
Consensus  mqaymeggqlgaclpsflvpdhyagfplplplqlpsqpnnklfqmpfdvnqeesenhggmlssdhcgglyplpalpfgscsgaaaatacg

ScWRKY3    GKPTAGFMPSAIGADEVCTSVATKLGCNESNSTWWKGSAATTAERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQKVVKNSLHP    180
SiWRKY12   GKPTADFMPSTVGADEVCTSLTK..GCNDSASTWWKGSAPTVADRGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQKVVKNSLHP    172
Consensus  gkptadfmpsaigadevctslakklgcndsastwwkgsaatmadrgkmkvrrkmreprfcfqtrsdvdvlddgykwrkygqkvvknslhp

ScWRKY3    RSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSADHTDCFSSF    236
SiWRKY12   RSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDAASGDHTDCFSSF    228
Consensus  rsyfrcthsncrvkkrverlstdcrmvmttyegrhthspcsddaasadhtdcfssf
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E

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ScWRKY3    .MQAYMEGGQLSACLPSFLVPDHYAGFPLPLPLQLPSQPNNKLFQMPFVVNQEETENHGGMLSSDHCGGLYPLPALPFGSCSGAAAATAC    89
OsWRKY12   MHTCMEGGQGTCLPNFYLLPDHHGMPLEPLQLPCHP..KLLQMPFDQEDQPGI.HGVMLSSDHC.GLYPLPALPLSNSAAAAAATVA    86
Consensus  mhqameeggqglaclpnflllddhagfplplplqlpchpnnklfqmpfdqedeegenhggmlssdhcgglyplpalpfgncaaaaaataa

ScWRKY3    GGKPT..AGFMPSAIGAEEVCTSVATKLGCNE.....SNSTWWKGSAATTAERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYGQK    172
OsWRKY12   LGKHSAAAGSMPNIGGAEEVATIV.TKAGNESTTCNGSTTWWRGSTMAAMGEKGKMKIRRKMREPRFCFQTRSEVDVLDDGYKWRKYGQK    175
Consensus  ggkhsaaagfmpnaggaeeevatsvatkagceettcngsnstwrggsaaamaekgkmkirrkmreprfcfqtrsdvdvlddgykwrkygqk

ScWRKY3    VVKNSLHPRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSADHTDCFSS    235
OsWRKY12   VVKNSLHPRSYFRCTHSNCRVKKRVERLSTDCRMVITTYEGRHTHSPC.DDNSSGBHTNCFSS    237
Consensus  vvknslhprsyfrcthsncrvkkrverlstdcrmvittyegrhthspcsddassadhtdcfss
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F

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ScWRKY3    MQAYMEGGQLSACLPSFLVPDHYAGFPLELPLQLPSQPNNKLFQMPFVVNQEETENHGGMLSSDHCGGLYPLPA.LPFGSCSGAAAATAC    89
ScWRKY4    ..MEGSSQLLETCLPASLYALSPCAPEPH.PLLAPLPNQHKLLQMPLL.VQEPAANNHGVMLFSDHHQHGCGPLYPLLLLFGICFCAAAAPAT    86
Consensus  mqaegegglleaclpaflvadhpaafphplpllaplpnnhklfqmpfvvneeaaenhggmlfsdhcggggplpalllfgicfcaaaapac

ScWRKY3    GGKPTAGFMPSAIGAEEVCTSVATKLG.........CNESNSTWWKGSAATTAERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYG    170
ScWRKY4    CEKTTTGFTALDAGEAGRTSAAKAAAEIASTTTTTCNGPSSCNWKGPAAAGEKCKMKVRRKMREPRFCFQTRSDVDVLDDGYKWRKYG    176
Consensus  cekptagffpaadaaeagctsaakaaaeiastttttcnepnscnwkggaaaaaekgkmkvrrkmreprfcfqtrsdvdvlddgykwrkyg

ScWRKY3    QKVVKNSLHPRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASSADHTDCFSS    235
ScWRKY4    QKVVKNSLHPRSYYRCTHSNCRVKKRVERLSEDCRMVITTYEGRHTHSPCSDDADAAAGDHTGS.    240
Consensus  qkvvknslhprsyfrcthsncrvkkrverlsedcrmvittyegrhthspcsddadaaagddcfss
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G

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ScWRKY3    ............MQAYMEGGQLSACLPSFLVPDHYAGFPLPLELQLPSQPNNKLFQMPFVVNQEETENHGGMLSSDHCGGLYPLPALPFG    78
Sc-WRKY    MAASLGLAHEACYAAYPPAAAASSYFPSPPPPGDLVAEPPPEAAATAMVDDYYFCFREEMGGARAPGCGGYCSPPAPVFDNGMNLLSYG    90
Consensus  maaslglaheacmaaymeaaaasacfpsflppdgdagaefplpaaapampddklfqfpeemggaeaegcggmcspdacgfdnglnalpfg

ScWRKY3    SCSGAAAATACGGKPTA..GFMPSAIGAEEVCTSVATKLGCNESNSTWWKGSAATTAERGKMKVRRKMREPRFCFQTRSDVDVLDDGYKW    166
Sc-WRKY    SADGCDGRRLMSCPAGGNGGCRPASLIG...........................................FRTRSDVDVLDDGFKW    133
Consensus  sadgaaaalacggaggagggfmasaigaeeevctsvatklgcnesnstwwkgsaattaergkmkvrrkmreprfcfqtrsdvdvlddgfkw

ScWRKY3    RKYGCKVVKNSLHPRSYFRCTHSNCRVKKRVERLSTDCRMVMTTYEGRHTHSPCSDDASS.....ADHTDCFSSF...............    236
Sc-WRKY    RKYGKKAVKSSPNPRNYYRCSAEGCCVKKRVERDRDDPRYVITTYDGVHNHAACGAAASCPPPRGADHTDCFSPFYSASSAPLVAAPSWN    223
Consensus  rkygkkavknslhprnyfrcsaegcgvkkrverdrddcrmvittydgrhnhaacgaaascpppprgadhtdcfspfysassaplvaapswn

ScWRKY3    ....................    236
Sc-WRKY    AAFDAWKAQLHAAAAHSSESS    244
Consensus  aafdawkaqlhaaaahssess
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Figure S5. Amino acid sequences alignment of ScWRKY3 and other WRKYs. A to G are showing the amino acid sequence alignment of ScWRKY3 and WRKY from *Sorghum bicolor* SbWRKY57 (XP_002452824.2), *Miscanthus lutarioriparius* MlWRKY12 (AGQ46321.1), *Zea mays ZmWRKY51* (XP_020393361.1), *Setaria italica* SiWRKY12 (XP_004953301.1), *Oryza sativa* OsWRKY12 (XP_015624962.1), *Saccharum* ScWRKY4 (AUV50355.1) and Sc-WRKY (ACT53875.1), respectively. All these accession numbers in brackets are from GenBank. The black and blue colors indicate the homology level of conservation of the amino acid residues in the alignment at 100 and ≥50, respectively.
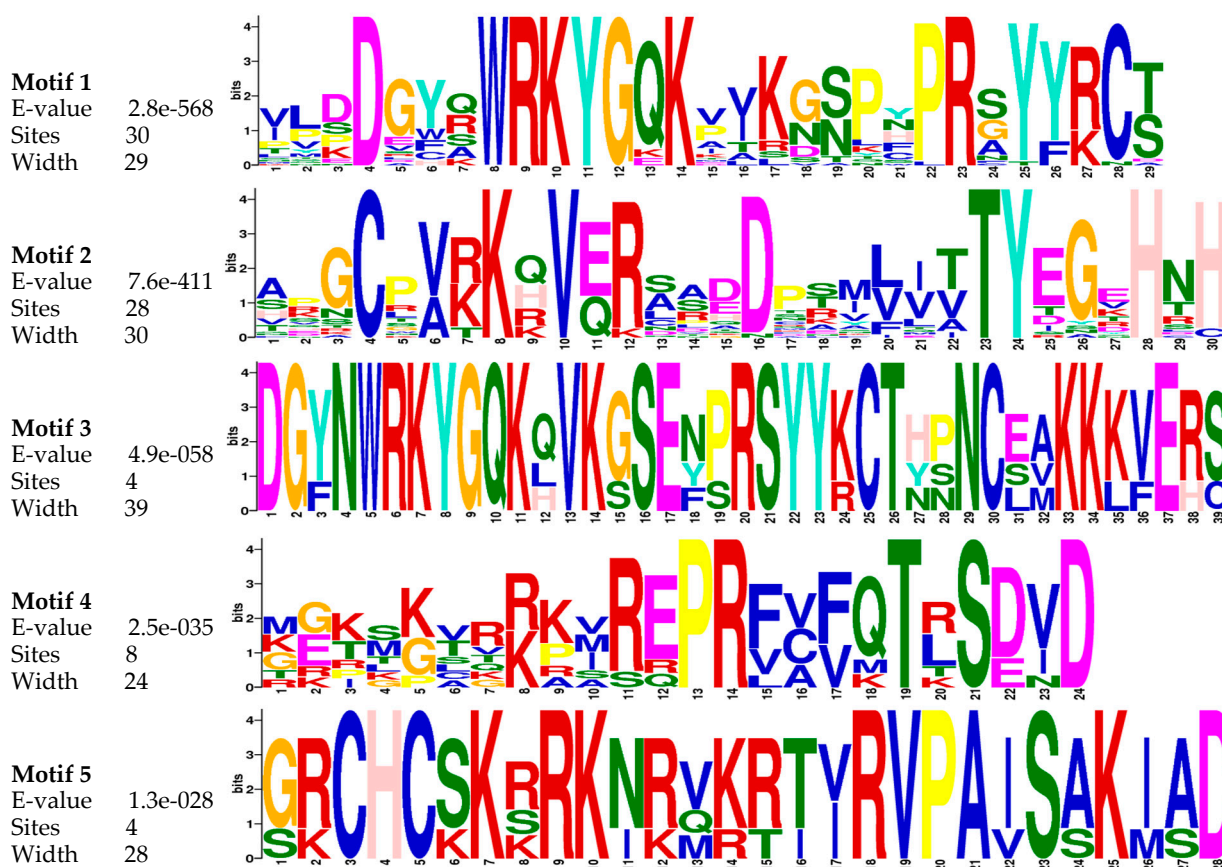
Motif 1		
E-value	2.8e-568	
Sites	30	
Width	29	

Motif 2		
E-value	7.6e-411	
Sites	28	
Width	30	

Motif 3		
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Sites	4	
Width	39	

Motif 4		
E-value	2.5e-035	
Sites	8	
Width	24	

Motif 5		
E-value	1.3e-028	
Sites	4	
Width	28	

Figure S6. The logo of predicted conserved motifs in the WRKYs. The logos were predicted by the online software MEME Suite 5.0.2 (http://meme.sdsc.edu/meme/intro.html). On the y axis (measured in bits), the overall height of the stack indicating the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino acid at that position.